mortality than at 2 months or later. At 2 months they often die suddenly without clear symptoms of HF, whereas at 3 months, many of them showed evident symptoms of HF. In isolated left ventricular myocardium (LV) from 2 month-mice, spontaneous activity frequently occurred and action potential duration was prolonged. Transient outward (I_{to}) and ultrarapid delayed rectifier K^+ currents (I_{Kur}) were significantly reduced in DCM myocytes. Correspondingly, down-regulation of Kv4.2, Kv1.5 and KChIP2 was evident in mRNA and protein levels. In 3 month-LV, more frequent spontaneous activity and further down-regulation in above K^+ channels were observed. 1 month mice, on the contrary, showed infrequent spontaneous activity in LV, in which Kv4.2 but not Kv1.5 or KChIP2 was down-regulated. Because they are at low risk of death in spite of enlarged hearts, reduction in Kv4.2 is not sufficient for sudden death (SD). Our results suggest that the combined down-regulation of Kv4.2, Kv1.5 and KChIP2 prior to the onset of HF may play an important role in the premature SD in this DCM model.

1729-Pos Board B499

Mitochondrial Oxidative Stress Mediates the Effect of Angiotensin II on Gap Junctional Remodeling and Sudden Arrhythmic Death

Ali A. Sovari[1], Euy-Myoung Jeong[1], Divya Arasu[1], Cody A. Rutledge[1], Elena Dolmatova[2], Nooshin Vahdani[1], Lianzhe Gu[1], Heather Duffy[2], Marcelo G. Bonini[1], Samuel C. Dudley[1].
[1]UIC, Chicago, IL, USA, [2]BIDMC-Harvard Medical School, Boston, MA, USA.

Introduction: Angiotensin II activation and associated elevation in ROS have been implicated in pathogenesis of arrhythmia. We created a transgenic mouse model of cardiac restricted overexpression of ACE (ACE8/8). These mice show spontaneous VT/ VF, SCD, and a reduction in Cx43 level, which impairs conduction and predisposes to arrhythmia. We sought to determine the role and the major source of ROS by angiotensin II in VT/ VF and Cx43 remodeling.
Method: Wild type and ACE8/8 mice with and without 2 weeks of treatment with LNIO (NOS inhibitor), Sepiapterin (precursor of BH4), Mito-TEMPO (mitochondria-targeted antioxidant), Apocynin (NADPH oxidase inhibitor), Allopurinol (Xanthine oxidase inhibitor), and ACE8/8 crossed with P67DN were studied. Western blotting, detection of mitochondrial ROS by MitoSOX red, electron microscopy, immunohistochemistry staining, and a fluorescent dye diffusion technique for functional assessment of Cx43 were performed. EP study was performed by a 1.1F catheter through pacing the right ventricle.
Results: Treatment with Mito-TEMPO prevented SCD in ACE8/8 mice ($p=0.0005$, 95% CI of 1.96 to 11.53). Treatment with Mito-TEMPO was also associated with reduction in VT inducibility (from 87% to 50%), correction of gap junction dye conduction (from 75% of control to normal, $P<0.05$), reduction of mitochondrial ROS (from 6 to 1.8 fold of the control), improvement of structure of mitochondria (detected by electron microscopy), increase in Cx43 level at the gap junctions (from 33% to 70% of control level detected by western blot and immunohistochemistry). Treatments with L-NIO, Sepiapterin, Allopurinol, Apocynin and crossing with P67DN mice did not prevent VT/VF and SCD in ACE8/8 mice.
Conclusion: Mitochondrial oxidative stress plays a central role in ROS-induced arrhythmia and mitochondria-targeted antioxidants may be effective antiarrhythmic drugs.

TRPV, TRPM, TRPP Channels

1730-Pos Board B500

Optical Recording of Single Channel TRPV1 Activity in Living Cells

Eric N. Senning, Sharona E. Gordon.
University of Washington, Seattle, WA, USA.

The multi-modal cation channel transient receptor potential vanilloid 1 (TRPV1), which is activated by heat, protons and a variety of endogenous and exogenous ligands amongst other stimuli, senses the extracellular environment. To investigate how the cell tunes its sensitivity to this environment, we asked whether the cell imposes functional constraints on TRPV1 by exercising a control over its localization. We and others have previously shown that TRPV1 channels can be regulated by trafficking and that surface expression of the channel is increased by treatment with Nerve Growth Factor (NGF), which induces inflammation. Investigation of fluorescently-labeled single channels by total internal reflection fluorescence (TIRF) imaging reveals a broad distribution of channel movement in the plasma membrane from immobile up to a lateral diffusion of 1.9 micrometer^2/sec. We next assayed the functional subset of this large distribution by sparklet analysis, which employs an intracellular fluorescent calcium indicator as a probe for TRPV1 opening events. TRPV1 sparklets appear as both mobile and immobile. Mobile TRPV1 sparklets exhibit an average effective diffusion of 0.072 micrometer^2/sec (n=9) with a maximal effective diffusion of .15 micrometer^2/sec for one channel. The time dependent behavior of the more mobile subset of sparklets reveals that their mobility decreased, and our ongoing effort to understand the spatio-temporal behavior of TRPV1 is directed at how the channel's activity slows its movement. Funding provided through the University of Washington Training Grant in Cardiovascular Pathology (NIH) and the National Eye Institute (NIH).

1731-Pos Board B501

Bradykinin Sensitization of TRPV1 Channels

Ivan F. Gonzalez, Jose L. Mercado, Carmen A. Ufret-Vincenty, Sharona E. Gordon.
University of Washington, Seattle, WA, USA.

Bradykinin (BK) and nerve growth factor (NGF) both sensitize TRPV1 channels in dorsal root ganglia (DRG) and trigeminal ganglia (TG) neurons of mammals. BK treatment of dissociated DRG neurons and transiently transfected cells expressing TRPV1 and the B_2 receptor for BK (a $G\alpha q$-coupled GPCR) confirmed reports in the literature of increased capsaicin-activated currents in whole-cell recordings. Calcium imaging of human embryonic kidney 293 cells (293T) and DRG-neuroblastoma hybrid cells (F-11) show a consistent calcium rise upon treatment with BK, but up to three-day-old dissociated DRG neurons seldom show a calcium rise. Confocal imaging of F11 and 293T cells transfected with a fluorescent diacylglycerol probe (C1-PKC-GFP) shows an increase of DAG in the plasma membrane (PM) upon application of BK, but a fluorescent phosphatidylinositol 4,5-bisphosphate (PIP_2) probe (YFP-PLCd1-PH) did not reveal a change in the PIP_2 levels on the PM. Calcium imaging of dissociated DRG neurons and F-11 cells expressing NGF-receptors trkA and p75 did not show calcium rise upon application of NGF. F-11 cells expressing NGF receptor, TRPV1 channels and a fluorescent phosphatidylinositol 3,4,5-triphosphate (PIP_3) probe (YFP-GRP1-PH) exhibit increased PIP_3 levels on the PM upon NGF treatment. F-11 cells expressing the PIP_3 probe did not show a change of PIP_3 levels on the plasma membrane upon application of BK. These results suggest different pathways for BK and NGF sensitization of TRPV1 channels in cultured cells. BK produces TRPV1 sensitization with no measurable change in PIP_2 levels, a rise in calcium and an increase on DAG, suggesting changes of second messengers downstream from phospholipase C (PLC) activation different from PIP_2, while NGF produces TRPV1 sensitization with a measurable change of PIP_3, and no significant change in calcium suggesting changes of second messengers downstream from phosphatidylinositol 3-kinase (PI3K). Funding provided by Grant EY07031 from the NEI.

1732-Pos Board B502

Molecular Organization of the Terminal Domains of the TRPV1 Ion Channel Determined by FRET Spectroscopy

Victor De la Rosa, Gisela E. Rangel, Tamara Rosenbaum, Leon D. Islas.
UNAM, Mexico, Mexico.

The TRPV1 ion channel is responsible for the perception of high temperatures, low pH and responds to binding of some pungent compounds. It is also associated with the perception of pain and noxious stimuli.
Fluorescent resonance energy transfer is a distance-dependent interaction between two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule.
By using FRET measurements between donor and acceptor pair introduced in the channel, we attempt to discern the molecular organization of the N- and C- termini of the TRPV1 ion channel. We genetically attached the YFP or CFP to the N- or C- termini of the protein, transfected the chimeras into HEK293 cells and measured FRET by the spectral FRET method. We were able to determine the architecture of the N and C termini within the tetramer, and its localization relative to the plane of the membrane.

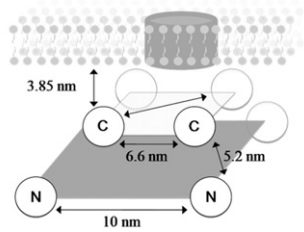

Figure 1. Molecular organization of the intracellular N- and C- termini of the protein.

1733-Pos Board B503

TRPV1 is Directly Activated by the Bioactive Lipid Lysophosphatidic Acid

Tamara Rosenbaum[1], Itzel Llorente[2], Andrés Jara-Oseguera[3], Diana Escalante-Alcalde[2], LEÓN D. ISLAS[3].
[1]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, D.F, Mexico, [2]Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, D.F., Mexico, [3]Facultad de Medicina, Universidad Nacional Autónoma de México, D.F., Mexico.

The bioactive phospholipid, lysophosphatidic acid (LPA), whose levels are increased upon tissue injury, activates primary nociceptors resulting in neuropathic pain. The TRPV1 ion channel is expressed in primary afferent nociceptors and is activated by physical and chemical stimuli. Our data show that LPA produces acute pain-like behaviors in control mice, which are substantially reduced in Trpv1 null- animals and that LPA activates TRPV1 through a novel mechanism that is independent of G protein-coupled receptors, contrary